

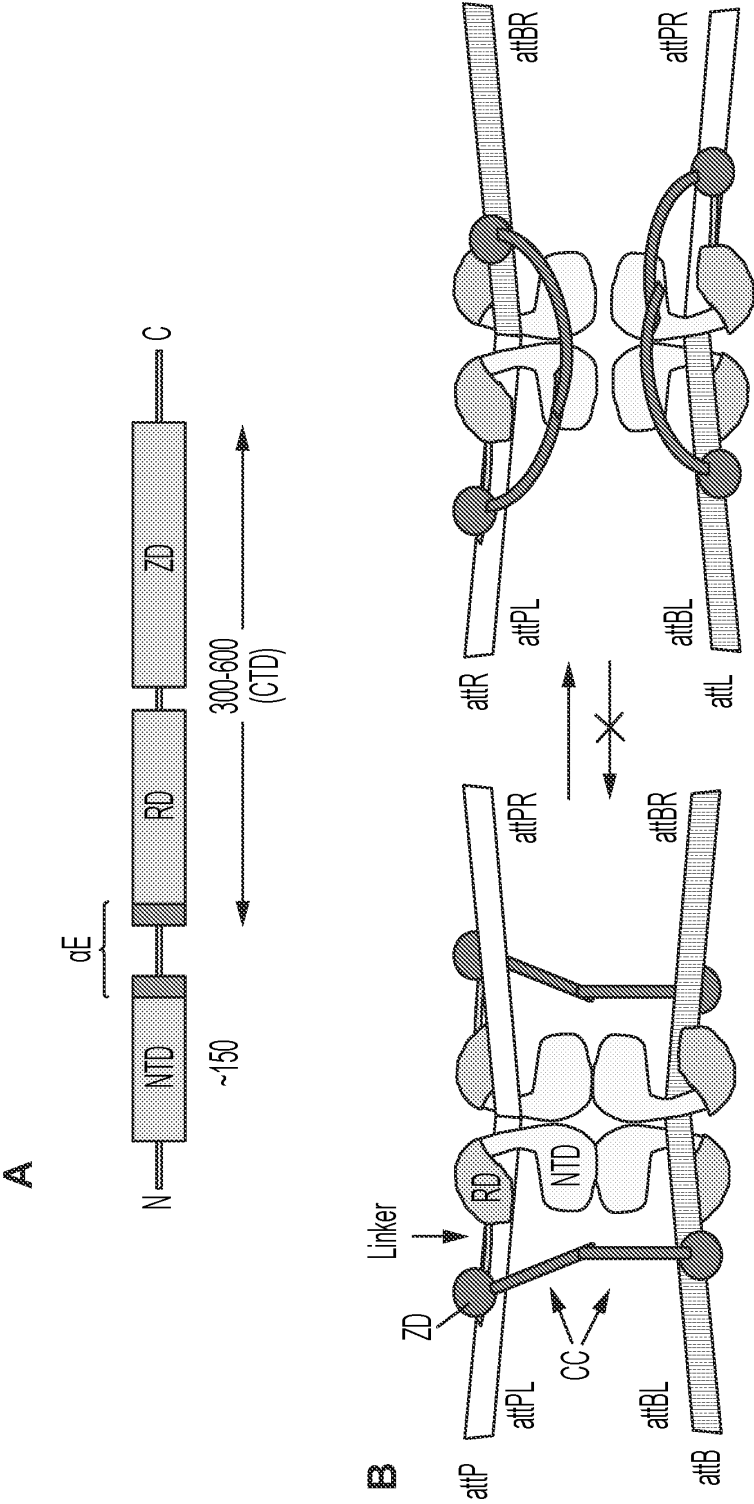


FIG. 1

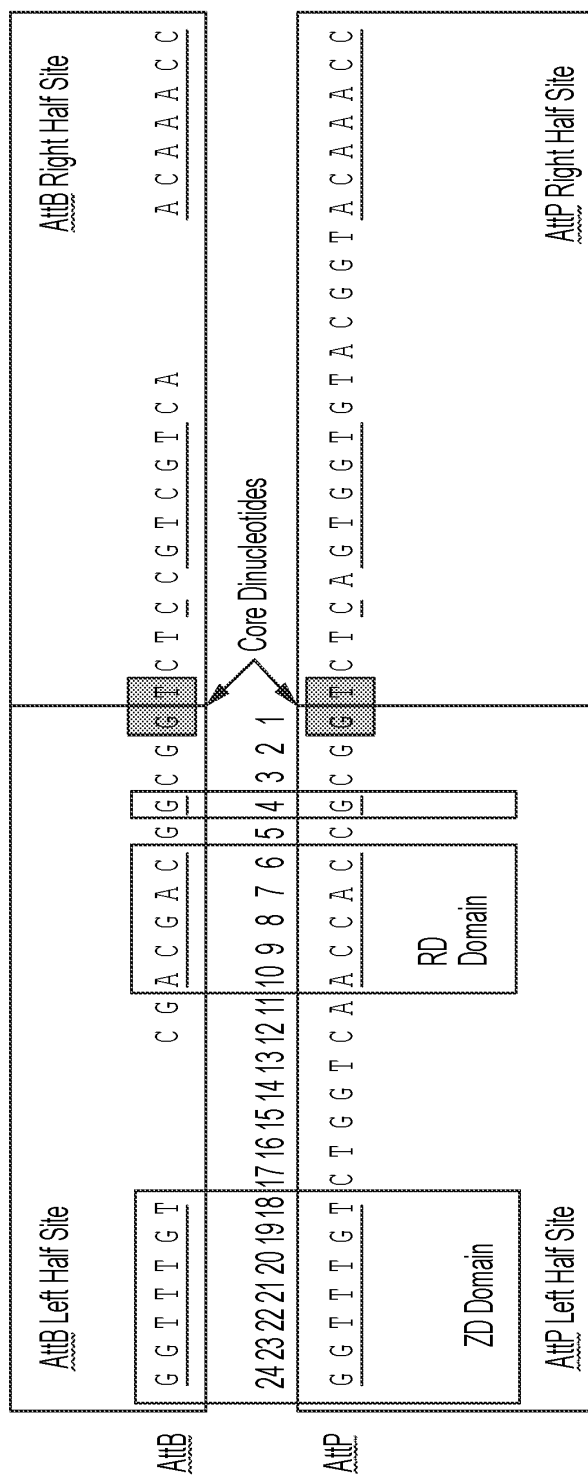
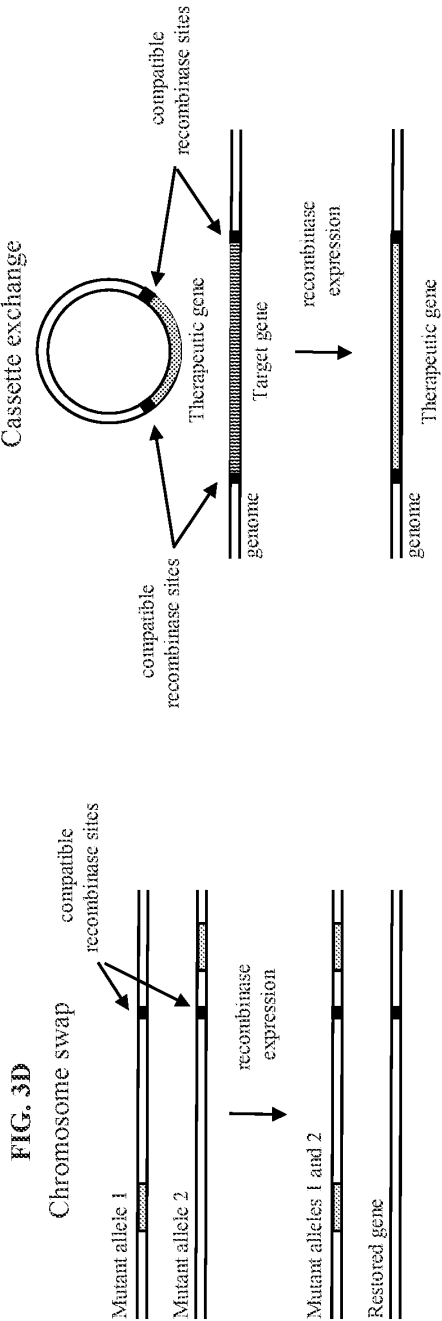
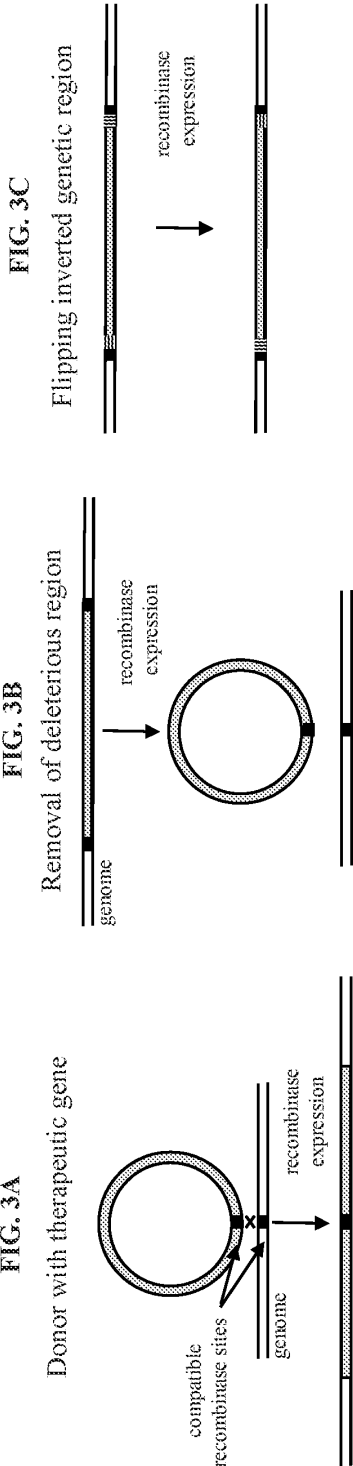


FIG. 2



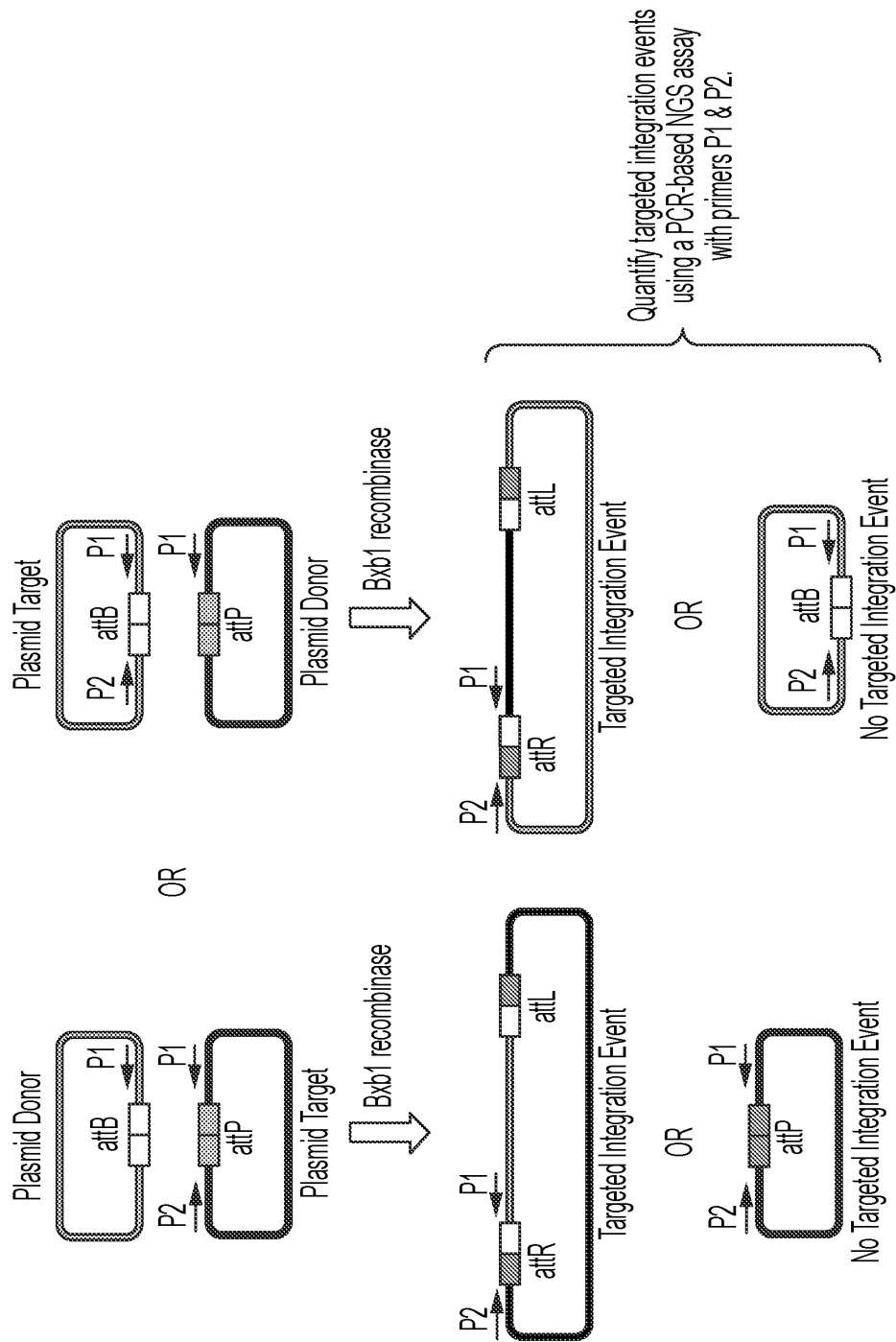


FIG. 4

5/11

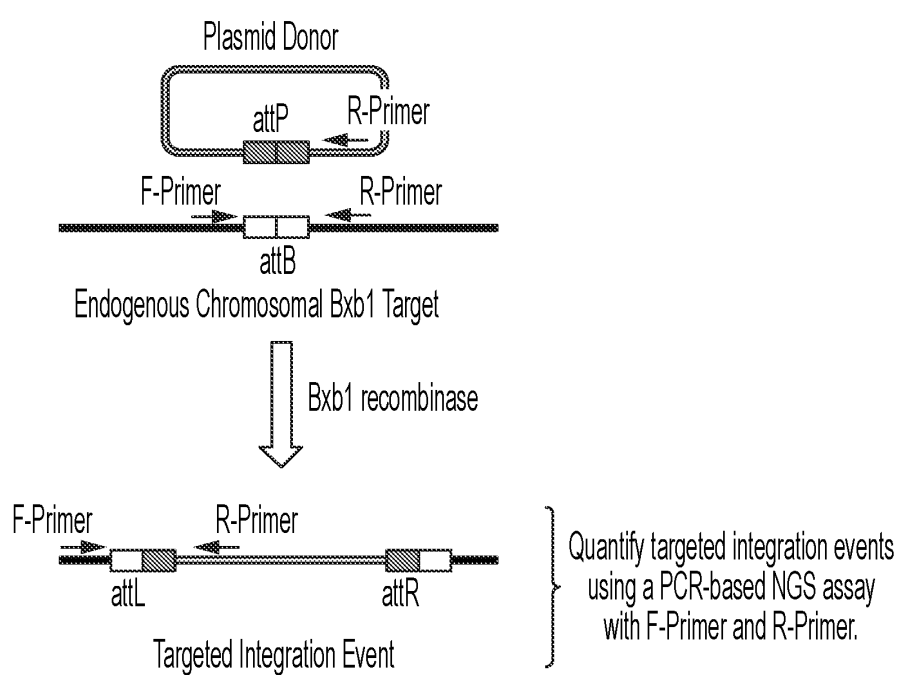


FIG. 5

FIG. 6A

position 10 (A in wt Bxb1 target)				
	%A	%C	%G	%T
WT	85.1	0.8	10.0	4.1
S231F	62.7	12.1	5.2	19.9

FIG. 6B

	Left half-site	Right half-site
s5-8	5'-GAGTAGTGATCTGACACACAGCACTCTCTGGTGCAGAACTCTTGGGTTCTCATCACATAGACTGTGGTCGTCTGAGACCCACAGTCTTAGAACCCAA-5'	
s5-1	5'-CCATTCTCTTCTACACACAGCGTTCTCAATTGGGTAAGCATCAAAATGGTAAGGAAGAAGATGGTGTCCCTGAGAGTTAACCCATTCTGTAGTTTA-5'	
s5-11	5'-TTGAAGCCCCCTTCTCCTACAGAGAGAGACAGCAGGGTAAATTTCTGTATTAACCTTCGGGGAGAGGATGTCTCTCTCGTCTGCCATTTAAGACATAA-5'	
s1-41	5'-CGAGCTTGCTCCTCACACAGAGAGCTCTCTGAAGACAGGAGCATCATGAGCTCGAACGAGGAGTGTCTCTCTGAGAGACTTCTGTCTCTCGTAGTACT-5'	

FIG. 6C

site	Mutations at Position 10	%targeted integration		S231F / wt
		S231F	wt Bxb1	
s5-8	None- matches wt Bxb1	6.95	6.92 +/- 2.25	1.00
s5-1	C in right halfsite	13.18	6.27 +/- 1.88	2.10
s5-11	C in both halfsites	9.46	1.59 +/- 0.82	5.95
s1-41	T in right halfsites	19.40	2.45 +/- 0.89	7.92

site	position	mutations in halvesites		Bxb1 variant	%TI		variant/wt
		left	right		variant	wt Bxb1	
s5-4	7	G	C	L158D	0.35	0.26 +/- 0.02	1.33
s5-4	7	G	C	T233F	0.66	0.26 +/- 0.02	2.51
s5-4	7	G	C	T233Y	0.88	0.26 +/- 0.02	3.34
s5-18	9	wt Bxb1	A	R237A	0.78	0.25 +/- 0.06	3.15
s5-18	9	wt Bxb1	A	R237C	0.51	0.25 +/- 0.06	2.06
s5-18	9	wt Bxb1	A	R237K	0.59	0.25 +/- 0.06	2.38
s5-18	9	wt Bxb1	A	R237N	0.57	0.25 +/- 0.06	2.30
s5-6	9	T	wt Bxb1	R237N	0.79	0.26 +/- 0.13	3.04
s3-28	10	C	wt Bxb1	N148T	0.41	0.25 +/- 0.05	1.62
s5-4	10	wt Bxb1	T	P197H	0.58	0.26 +/- 0.02	2.20
s3-28	10	C	wt Bxb1	S231F	0.42	0.25 +/- 0.05	1.66
s5-1	10	wt Bxb1	C	S231F	13.18	6.27 +/- 1.88	2.10
s5-11	10	C	C	S231F	9.46	1.59 +/- 0.82	5.95
s5-4	10	wt Bxb1	T	S231F	1.60	0.26 +/- 0.02	6.08
s5-19	10	T	G	S231F	23.59	2.11 +/- 0.56	11.18
s1-41	10	wt Bxb1	T	S231F	19.40	2.45 +/- 0.89	7.92
s5-4	10	wt Bxb1	T	S231G	0.76	0.26 +/- 0.02	2.89
s5-4	10	wt Bxb1	T	S231R	0.43	0.26 +/- 0.02	1.63
s1-41	10	wt Bxb1	T	S231R	5.77	2.45 +/- 0.89	2.36
s5-11	10	C	C	S231Y	6.00	1.59 +/- 0.82	3.77
s3-28	10	C	wt Bxb1	S231V	0.40	0.25 +/- 0.05	1.58
s5-4	10	wt Bxb1	T	S231V	0.57	0.26 +/- 0.02	2.16
s5-4	10	wt Bxb1	T	T233F	0.66	0.26 +/- 0.02	2.51
s1-1	10	C	wt Bxb1	T233K	0.10	0.04 +/- 0.02	2.43
s1-5	10	G	wt Bxb1	T233R	0.09	0.03 +/- 0.02	2.94
s3-28	10	C	wt Bxb1	T233W	0.39	0.25 +/- 0.05	1.54
s5-4	10	wt Bxb1	T	T233W	1.55	0.26 +/- 0.02	5.89
s5-19	10	T	G	T233W	5.21	2.11 +/- 0.56	2.47
s5-4	10	wt Bxb1	T	T233Y	0.88	0.26 +/- 0.02	3.34

FIG. 7

position 19 (G in wt Bxb1 target)					position 21 (T in wt Bxb1 target)				
	%A	%C	%G	%T		%A	%C	%G	%T
WT	0.8	1.8	96.5	1.0	WT	2.2	0.1	12.5	85.2
F314G	0.7	5.3	73.7	20.3	G316Y	4.8	0.2	78.5	16.6

FIG. 8A

	Left half-site	Right half-site
Symmetric AttB site	5'-GGTTTGTGAGACGGCGCTCTCCGTCGTCAACAAACC CCAAACAGCTGTGTCGCCGCCGAGGCAGTGTGTTGG-5'	
s5-16	5'-GCACTCCAGCCTGGGCAACAGAGCCTCTGTGTTGTCAAAAAA CGTGAGGTCGGACCCGTTGTCTCTGAGACAACAGTTT-5'	Position 19 T
s3-28	5'-ACCTTTGTTTGGCCCAACTGCCCTGTGCCACTGTGCACACACTTGGGA TGGAAACAAACCGGGTTGACGGGACGGTGACAGCTGTGTGAACCCCT-5'	Position 21 G

FIG. 8B

site	Mutations at Position 19	%targeted integration F314G	wt Bxb1	F314G / wt
s5-16	T in right half-site	3.11	1.09 +/- 0.28	2.85

site	Mutations at Position 21	%targeted integration G316Y	wt Bxb1	G316Y / wt
s3-28	G in right half-site	0.45	0.25 +/- 0.05	1.78

FIG. 8C

site	position	mutations in halvesites		Bxb1 variant	%TI		variant/wt
		left	right		variant	wt Bxb1	
s5-24	19		T	F314A	0.21	0.12 +/- 0.03	1.75
s5-6	19		T	F314C	0.66	0.26 +/- 0.13	2.54
s5-16	19		T	F314C	2.21	1.09 +/- 0.28	2.03
s1-5	19	wt Bxb1	T	F314C	0.15	0.03 +/- 0.02	4.97
s5-6	19		T	F314G	1.84	0.26 +/- 0.13	7.08
s5-16	19		T	F314G	3.11	1.09 +/- 0.28	2.85
s5-21	19	T		F314N	1.74	0.85 +/- 0.15	2.04
s5-21	19	T		F314Q	1.78	0.85 +/- 0.15	2.09
s5-6	19		T	F314Q	0.71	0.26 +/- 0.13	2.73
s5-16	19		T	F314Q	3.21	1.09 +/- 0.28	2.94
s1-5	19	wt Bxb1	T	F314Q	0.12	0.03 +/- 0.02	4.19
s5-6	19		T	F314S	2.03	0.26 +/- 0.13	7.81
s1-5	19	wt Bxb1	T	F314S	0.14	0.03 +/- 0.02	4.66
s5-6	19		T	F314T	2.40	0.26 +/- 0.13	9.23
s5-16	19		T	F314T	2.27	1.09 +/- 0.28	2.08
s1-5	19	wt Bxb1	T	A315F	0.12	0.03 +/- 0.02	3.92
s5-6	19		T	A315I	0.85	0.26 +/- 0.13	3.27
s1-5	19	wt Bxb1	T	A315I	0.11	0.03 +/- 0.02	3.61
s5-16	19		T	A315S	2.79	1.09 +/- 0.28	2.56
s1-5	19	wt Bxb1	T	A315S	0.08	0.03 +/- 0.02	2.60
s5-24	19		T	A315W	0.24	0.12 +/- 0.03	2.00
s5-16	19		T	A315Y	2.11	1.09 +/- 0.28	1.94
s5-16	19		T	G318R	2.17	1.09 +/- 0.28	1.99

FIG. 9

site	position	mutations in halvesites		Bxb1 variant	%Tl		variant/wt
		left	right		variant	wt Bxb1	
s5-6	21		C	F314E	1.06	0.26 +/- 0.13	4.08
s3-28	21	wt Bxb1	G	G316Y	0.45	0.25 +/- 0.05	1.78
s1-5	22	G	A	A315F	0.12	0.03 +/- 0.02	3.92
s1-5	22	G	A	A315H	0.16	0.03 +/- 0.02	5.27
s1-5	22	G	A	A315N	0.13	0.03 +/- 0.02	4.32
s5-24	22		A	A315W	0.24	0.12 +/- 0.03	2.00
s1-5	22	G	A	G316A	0.11	0.03 +/- 0.02	3.82
s1-5	22	G	A	G316C	0.14	0.03 +/- 0.02	4.86
s1-11	23	T	A	A315F	0.11	0.04 +/- 0.01	3.19
s1-11	23	T	A	A315H	0.35	0.04 +/- 0.01	9.87
s1-11	23	T	A	A315W	0.12	0.04 +/- 0.01	3.30
s1-11	23	T	A	A315Y	0.30	0.04 +/- 0.01	8.28
s5-21	23	T		G316C	1.47	0.85 +/- 0.15	1.72
s1-10	23	T	T	G316C	1.57	0.49 +/- 0.15	3.20
s1-11	23	T	A	G316C	0.08	0.04 +/- 0.01	2.35
s1-11	23	T	A	G316T	0.18	0.04 +/- 0.01	4.98
s1-11	24	C	wt Bxb1	A315F	0.11	0.04 +/- 0.01	3.19
s1-11	24	C	wt Bxb1	A315Y	0.30	0.04 +/- 0.01	8.28

FIG. 10

Site	100% variant		50% variant + 50% wt	
	D257K	wt Bxb1	D257K	D257K/wt
s1-10	1.37	0.35 +/- 0.04	0.96	0.49 +/- 0.15
s1-39	6.70	0.48 +/- 0.09	2.10	0.64 +/- 0.16
s3-28	1.94	0.38 +/- 0.19	0.46	0.25 +/- 0.05
s3-41	2.66	0.41 +/- 0.04	1.29	0.32 +/- 0.17
s5-10	2.06	0.28 +/- 0.03	1.51	0.39 +/- 0.12
s5-11	10.03	1.11 +/- 0.41	1.67	1.59 +/- 0.82
s5-14	2.78	0.29 +/- 0.10	0.70	0.36 +/- 0.24
s5-15	3.38	0.72 +/- 0.25	1.07	1.49 +/- 0.61
s5-16	5.79	1.33 +/- 0.63	2.38	1.09 +/- 0.28
s5-17	2.55	1.23 +/- 0.42	1.32	1.13 +/- 0.68

FIG. 11